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Swine Influenza Virus Vaccine: Potentiation in Rhesus Monkeys of
Antibody Responses by a Nuclease Resistant Derivative of
Poly I-Poly C

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The USAMRIID facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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ABSTRACT

Polyriboinosinic-polyribocytidylic acid stabilized with poly-l-lysine and carboxymethylcellulose (poly ICLC) enhances the antibody response in rhesus monkeys immunized with swine influenza virus subunit vaccine. Monkeys given the vaccine-adjuvant combination had earlier and significantly ($P < 0.05$) higher titers by 14 days. The potentiation of the antibody response of young monkeys given a split-virus vaccine in combination with poly ICLC suggests that this vaccine adjuvant combination may similarly provide a potentially useful alternative approach to the immunization against swine influenza of the pediatric and young adult age groups.

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Parkman et al. (1) have stated that single doses of the influenza A/NJ/76 virus vaccines are less than satisfactory for immunization of persons below age 25 against swine influenza. Several alternative approaches to immunize the pediatric age group are under consideration including the use of lower doses of the whole virus vaccine, splitvirus vaccines with more antigen and two-dose sequences (1). Another alternative possibility would be to employ an adjuvant to increase the potency of the vaccine. Hilleman (2) reported that a complex of polyriboinosinic and polyribocytidylic acids (poly I·poly C) caused comparatively weak potentiation of antibody response to ordinary aqueous influenza vaccine. Poly I·poly C, however, is only minimally effective in primates as an interferon inducer, possibly because of the presence in primate serum of high concentrations of nucleases that hydrolyze the compound. A complex of poly I·poly C with poly-l-lysine and carboxymethylcellulose (poly ICLC) has been shown to be a much more effective interferon inducer in primates than the parent compound (3). In addition, poly ICLC significantly enhances the antibody response of rhesus monkeys to formalin-inactivated Venezuelan equine encephalomyelitis virus vaccine (4). The present report presents data to show that poly ICLC potentiates the antibody response to a monovalent influenza subunit antigen prepared from the A/New Jersey/76 (swine) strain of virus, when tested in monkeys.

Monovalent influenza virus subunit vaccine, designated A/Swine X-53, lot #17061, (kindly supplied by Wyeth Laboratories, Inc., Philadelphia, Pa) was used. The dosage of poly ICLC, prepared as described previously (3), was either 0.1 or 0.3 mg/kg. Poly ICLC was combined with the vaccine just prior to immunization and given in the gracilis muscle. Hemagglutination inhibition (HAI) titers were done by the method

described by Robinson and Dowdle (5) as adapted for microtiter technique. The antigen used in the HAI tests was prepared using the A/Swine X-53 strain of influenza virus provided by the Center for Disease Control (5). Sixteen healthy, well-conditioned, young adult male or female rhesus monkeys (Macaca mulatta) weighing 4 to 7 kg were used in the study, and placed into 4 groups of 4 monkeys each. One group was used as controls. In the other groups, the vaccine (200 CCA units/monkey) was combined with either poly ICLC (0.1 or 0.3 mg/kg) or an equivalent volume of saline so that each monkey was injected intramuscularly with 0.8 ml total volume. A negative control group was given an equivalent volume of saline alone without vaccine. The monkeys were bled prior to inoculation and on days 7, 14, and 28 after inoculation for antibody determinations. In the calculation of geometric mean HAI antibody titers, negative responses were assigned values which were one-half of the lowest detectable titer of 1:10. Rectal temperatures were recorded twice each day. Temperatures above 39.7 C were considered a febrile response.

The HAI antibody responses of monkeys given one dose of vaccine alone or in combination with the adjuvant poly ICLC are shown in Table 1. Monkeys given either 0.1 or 0.3 mg/kg of poly ICLC in combination with vaccine had significantly greater ($P < 0.05$) antibody responses at 14 and 28 days after inoculation than monkeys given the vaccine alone. Only 1 of 4 monkeys given the vaccine without poly ICLC had detectable HAI antibody by day 14 (titer of 1:20); whereas, 8 of 8 monkeys given vaccine with poly ICLC had titers of 1:20 or greater and 6 of 8 had titers of 1:40 or greater. By day 28, 8 of 8 poly ICLC-treated monkeys

had titers greater than or equal to 1:40. There was no difference between adjuvant doses in their effect on the antibody response of monkeys.

Fever was not observed in monkeys given either saline or vaccine alone. In monkeys given vaccine in combination with poly ICLC, 3 of 8 monkeys had rectal temperatures greater than 39.7 C (40.0, 40.0 and 40.3 C) only at 24 hours after vaccination. Two of the three febrile monkeys were given 0.3 mg/kg of the poly ICLC. By 48 hours after injection, none of the monkeys had fever. There was no induration or erythema at the injection site in any of the vaccinated monkeys.

Potential of the weakly immunogenic subunit influenza virus vaccine, in addition to potentiation of killed Venezuelan equine encephalomyelitis whole virus vaccine (4), suggests that poly ICLC may have potential for widespread use as an immunological adjuvant for weakly antigenic vaccines. In some circumstances inactivated virus vaccines, though less antigenic, may offer advantages over live virus vaccines, such as increased short and long-term safety, reduced reactogenicity, and improved control of production methodology.

Poly ICLC has been given experimentally to human patients and is known to cause moderate febrile responses (6). If present adjuvant studies are extended to include man, the lowest possible dosages should be used to decrease the volume required for an injection and the potential for nonspecific febrile reactions.

References and Notes

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7. We thank W. R. Beisel, R. E. Edelman, D. G. Harrington and H. Rozmiarek for criticism of the manuscript.

Table 1. HAI titer response of 4 individual monkeys per group given 200 CCA units of influenza vaccine (A/Swine X-53) with or without poly ICLC as an adjuvant.

Treatment		Reciprocal antibody titer by days after vaccination (Values in parenthesis represent group geometric means)			
Vaccine	Poly ICLC (mg/kg)	0	7	14	28
-	-	<	<	<	<
		<	<	<	<
		<	<	<	<
		<	<	<	<
+	-	<	<	<	<
		<	<	< (7)	< (12)
		<	<	<	20
		<	<	20	40
+	0.1	<	<	20	40
		<	< (8)	40	40
		<	10	80 (80)*	160 (95)*
		<	20	640	320
+	0.3	<	<	20	40
		<	< (7)	40	160
		<	<	40 (48)*	160 (113)*
		<	20	160	160

< = HAI titers below the lowest detectable value of 1:10.

*Geometric mean titers are significantly different ($P < 0.05$) when compared to the vaccinated group of monkeys that received no poly ICLC.

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alternative approach to the immunization against swine influenza of the pediatric and young adult age groups.

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